Leaf assisted bioreduction of silver ions using leaves of *Centella asiatica L* and its bioactivity

[1]Palaniselvam K., [2*]A.Antony joseph velanganni., [3]S.Natanamurugaraj Govindan and [4]Karthi

1. Department of Biochemistry, School of Life Science,
   J.J college of arts and science, Pudukkottai-622422. Tamilnadu, India.
2. Department of Biochemistry, Bharathidasan University,
   Thiruchirapalli-620024, Tamilnadu India.
3. Department of Industrial science and Technology,
   University of malaysia Pahang, Malaysia-26300.
4. Department of Nanoscience and Technology, Bharathiyar University,
   Coimbatore-641114 Tamilnadu India.

* Corresponding author Address:Ph: 9942337107
E.mail: velanganni80@gmail.com. (A.Antony joseph Velanganni)

Abstract

In the present study to check potential effect of antimicrobial activity and leaf assisted bioreduction of silver ion using *Centella asiatica* has been studied. The leaves of *Centella asiatica* are used for antimicrobial activity and biosynthesis of silver nanoparticles. Instead the *C. asiatica* analysis of phytochemicals revealed the presence of alkaloids, flavanoids, tannins etc., On treatment of aqueous solution of silver nitrate with the leaf extract of c.asiatica the rapid formation of stable silver nanoparticles at high concentration is observed occur. The reduction of the metal ions and stabilization of silver nanoparticle is believed to occur by the terpenoid, flavonoid constituents of extract as well as by reducing sugar ascorbate and protein molecule in the broth. And also the plant extract were analysed antimicrobial activity of gram positive and gram negative bacteria using by agar well method supplementary 10µl, 20µl, 30µl and control which observed of result zone of inhibition. However finally conclude the promoising effect *Centella asiatica* leaf extract synthesis of silver nanoparticles are necessary to evaluate the positive use as a new bactericidal material.

Keywords : *Centella asiatica*, Antibacterial, Silver nitrate, bioreduction, silver nanoparticles.

1. Introduction

Synthesis of inorganic nanoparticle by biological systems makes more biocompatible and environmentally benign. These nanoscale matter going to play important area such as catalysis, optics, mechanics, magnetics and energy science (Govindaraju *et al.*, 2009). Beside these nanomaterial have variety of biomedical applications are treatment specific dermatological disease, surgery for using nanorobots and other drug formulation application (Sandi *et al.*,2004).

Use of the biological organisms such as micro organisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner (sastry *et al.*, 2004). Silver nanoparticles have many application for example they might be used as spectrally selective coating for solar energy absorption and interchelation material for electrical batteries, as optical receptors, as a catalysis in chemical reactions for biolabelling and as antimicrobial agents (Panalek A et al 2006). Several bacterial strains were reported as silver resistant and may even accumulate silver at the cell wall to as much as 25% of dry weight biomas, thus suggesting their use for the industrial recovery of silver from the material. Nanocrystals of gold, silver and their alloys were prepared by reaction of the corresponding metal ions with into felt of lactic

E - Journal of Life Science : www.e-journal.in

acid bacteria present in butter milk (Nair and Pradeep, 2002; Elumalai *et al.*,2010).

Fungal may be used to grow nanoparticles of different composition and size based on their exhaustive investigation, two different of fungi *verticillium sp and oxysporum* are the good candidate for the synthesis of nanomaterials. When the species are exposed to aqueous gold and silver ion reduce the metal ions pairly and rapidly. (Mukherjee *et al.,* 2001).

Centella asiatica L. commonly known as Indian pennywort belongs to family Apiaceae. Traditionally it is used as nervine tonic and for the treatment of asthma, hypertension, bronchitis, dropsy, skin diseases, and urethritis (Chakraborty T *et al.,*1996). *C. asiatica* has antibacterial, antifeedant, antituberculosis, antileprotic, and antioxidant properties (Tiwari Kn *et al.*,2000). Glycosides like indocentelloside, brahmoside, brahminoside, asiaticoside and theankuniside have been isolated from this plant. Asiaticoside is used in treatment of leprosy and uberculosis.

Extra cellular synthesis of pure metallic silver and gold nanoparticles, on treatment of aqueous solution of silver nitrate and chloroauric acid with neem leaf broth, for rapid formation of stable silver and gold nanoparticles. (Shankar *et al.*, 2004). Biogenic gold tranotriangles and spherical silver nano particles were synthesized by a single procedure using Aloe vera leaf broth as the reducing a agent reduction of silver ions by formation of spherical silver nanoparticles (Chandran., 2006). The aim of the work was to carry out synthesis of silver nanoparticles in leaves of *Centella asiatica* and its antibacterial activity.

2. Materials and methods

2.1. Plant Material

On the basis of the edible, which are shortly available in the literature in plant *Centella asiatica* were used for the analysis of antibacterial activity and to check the efficient candidate for the synthesis of silver nanoparticles. The plant materials were collected from shrubby forest in Arimalam, Pudukkottai, Tamilnadu, India.

2.2. Leaf broth preparation and synthesis of Ag Nanoparticles

Collected leaves are cleaned running tap water and shadow air dried for 6-10 days. The dried plant material were kept in the hot air oven at 40^0C for 5-6 hrs. The leaves were ground to a fine powder. 1mg of (w/v) 1mm silver nitrate was added to plant extract to make upto a final solution 200ml and centrifuged at 18,000 rpm for 25min. The collected pellets were stored at 4^0C. The supernatant was heated at 50^0C at 5 min. A change in the colour of solution was observed.

2.3. Biochemical Investigations

Biochemical investigation of leaf extract was made to check the possible bio-molecules involved in the reduction of silver ion to metal Ag. The plant leaves extract was check for reducing sugar by Benedicts method and protein by lowry using BSA as standard (Lowry 1955).And also check other phytochemical parameters such as alkaloids, flavonoids, saponins, glycosides and terpinoids(Horbone, 1984).

2.4. Spectral characterization

The reduction of Ag^+ ion into metallic silver nanoparticles was monitored by measuring the Uv-vis spectrum of the reaction medium at 5 hrs. after diluting of small aliquot of the sample into distilled water 0.1 ml respectively. UV-Vis spectral analysis was done by using UV-Vis spectrophotonuts UV-2450 (Shimadzu).

2.5. Particle Size characterization using SEM Analysis

Scanning electron microscope (SEM) analysis was done using Hitachi S 4500 SEM machine. This films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on tea sem grid were allowed to dry by putting it under a mercury lamp for 5 min.

2.6. Antibacterial Assay

Antibacterial activity of the synthesized silver nanoparticle were determine using the agar well diffusion method against pathogenic gram

E - Journal of Life Science : www.e-journal.in

negative and gram positive bacteria *E.coli, Bacillus subtilis, pseudomonas auroginosa, staphylococcus aures and klebselle pneumonia*. The pure culture of Bacteria were subculture on nutrient agar medium and broth respectively. Wells of 10mm diameter were made on nutrient agar plates using gel functure. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette different concentration of the sample of nanoparticle solution (10µl, 20µl and 30µl and control) was poured onto each well on all plates. After incubation at 37^0C for 24 hours. The different levels of zone of inhabitation of bacteria were measured by using zone reader.

3. Results and discussion

The present work demonstrates the formation of the silver nanoparticles by the reduction of Ag^+ ion using the plant extract *Centella asiatica*. Reduction of silver ion into Ag nano particle during exposure to the plant extract. *Centella asiatica* could be followed by colour change (Fig-1) The colourless solution changed into brownish yellow colour which indicates the formation of silver nanoparticles.

Formation of Ag nanoparticle was monitored by UV-Vis spectroscopy. UV-Vis spectrograph of the colloidal solution of Ag nanoparticles has been recorded as a function of time. (Fig 2) Absorption spectra of Ag nanoparticles formed in the reaction media at 10min has absorbance peak at 430nm broadening of peak indicated that the particles are ploy dispersed.

The SEM image are (not showed) relatively spherical shape nanoparticle formed with diameter range 50-60nm. Further, the nanoparticle synthesis by green route was found highly toxic against five isolated bacterial species at a various concentration of 10µl, 20µl, 30µl. 30µl Ag nanoparticles revealed higher antibacterial activity against *E.coli* and *S.aureus* where as intermediate activity was revealed against *B-subtilis*, *K.pneumoniae* and *P.aeuroginosa*. The inhibitory activities in culture media of the Ag nanoparticles reported in Tabl-1 where comparable with standard anti-microbics viz, chloromphenical.

The mechanism of the bactericidal effect of silver colloid particles against bacteria is not very well known (Ales., 2008). If silver nanoparticles may attach to the surface of the cell membrane and disturb its powder function such as permeability and respiration. It is reasonable to state that the binding of the particles to therapeutic value into the human body to perform cellular repairs at the molecular level(Huanget *et al.*, 2007; Raveendran *et al.*, 2006).

Recently much work has been done with regard to plant assisted reduction of metal nanoparticles and the respective role of phytochemicals. The main phytochemicals responsible have been identified as terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids in the light of IR spectroscopic studies. The main water soluble phytochemicals are flavones, organic acids and quinones which are responsible for immediate reduction. The phytochemicals present in *Bryophyllum sp*. (Xerophytes), *Cyprus sp.* (Mesophytes) and *Hydrilla sp.* (Hydrophytes) were studied for their role in the synthesis of silver nanoparticles. The Xerophytes were found to contain emodin, an anthraquinone which could undergo redial tautomerization leading to the formation of silver nanoparticles.(Govindaraju *et al.*,2010; Singaravelu G *et al.*, 2007).

4. Conclusions

The present investigation provides a environmentally benign protocol for the rapid synthesis of stable nanoparticle using leaf extract of *Centella asiatica*. Further the Ag nanoparticle revealed to possess an effective antibacterial property against gram negative and gram positive bacteria. The green chemistry approach to synthesis of silver nanoparticle have excellent antibacterial activity, and simple, cost effective and reproducible.

5. Reference

1. Ales Panacek., Libor Kvýtek., Robert Prucek., Milan Kolar., Renata Vecerova., Nadezda Pizurova., Virender, K. Sharma., Tat¢jana Nevecna and Radek Zboril., 2006. Silver Colloid Nanoparticles: Synthesis, Characterization, and their Antibacterial Activity. Journal of Physical Chemistry B, **110**: 16248-16253.

2. Chakraborty T, Sinha BS, Sukul NC., 1996. Preliminary evidence of antifilarial effect of *Centella asiatica* on canine dirofilariasis. Fitoterapia, 67:110-112.

3. Chandran SP., Chaudhary.M., M.Pasricha R., 2006. Synthesis of gold nano triangle and silver nano particles using Aloe vera plant extract. J. Biotechnol. Prog. (2) 537-543.

4. Elumalai E.K., Prasad, T.N.V.K.V., Hema Chandran J., Viviyan Therasa S., Thirumali T., and David E., 2010. Extra cellular synthesis of silver nanoparticle using leaves of Euphorbia hirta and their antibacterial activities. J.Pharm. Sci S. Res. Vol 2(9) : 549-554.

5. Govindaraju K., Tamil Selvans, Kiruthiga V., and singaravelu G., 2010. Biogenic silver nanoparticle by solanum turvum and their promising antimicrobial activity. Journal of Biopesticides (3) (1 special issue) 394-399.

6. Govindaraju, K., Kiruthiga, V., Ganesh Kumar, V. And Singaravelu, G., 2009. Extracellular synthesis of silver nanoparticles by a marine alga, *Sargassum wightii* Grevilli and their antibacterial effects. Journal of Nanoscience Nanotechnology, **9**: 5497-5501.

7. Huang,J., Li, Q., Sun, D., Lu,Y., Su,Y., Yang, X., Wang, H., Wang, Y., Shau, W., He, N., Hong, J. and Chen,C. 2007. Biosynthesis of silver and gold nanoparticles by novel sun dr eid *Cinnamomum camphora* leaf. Nanotechnology, **18**: 1-11.

8. Sharma KN, Tiwari V, Singh BD 2000. Micropropagation of *Centella asiatica* (L.), a valuable medicinal herb. Plant Cell Tiss Org, 63:179-185.

9. Lowry OH., Rosebrough N.J., Farr A.L., and Randall RJ 1951. Protein measurement with Folin Phenlo Reagent. Journal of Biochemistry Vol (193) : 265-275.

10. Mukherjee,P., Roy, M., Mandal, B., Dey, G., Mukherjee , P., Ghatak , J.,2008. Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus T. asperellum.Nanotechnology., 19, 75103.

11. Nair B., and Pradep T., 2002.Coatescence of nano clusters and formation of sub micron crystallites assisted by lacto bacillus strains. Journals of nanotechnology (2): 293-298.

12. Panacek A, Kvitek L, Prucek R, Kolar M, Vecerova R, Pizurova N., 2006. Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity. J Phys Chem B;110 (33):16248-16253.

13. Raveendran P, Fu J, Wallen SL., 2006. A simple and ‘‘green’’ method for the synthesis of Au, Ag, and Au-Ag alloy nanoparticles. Green Chem, 8: 34-38.

14. Sandi Salope K, and Sandi B., Silver 2004. Nanoparticles as antimicrobial agents, a case study on E-coli as a model for gram negative bacteria. Journal of Colloid and interface science vol (275): 177-182.

15. Sastry, M., Ahmad, A., Khan, M.I., and Kumar, R., 2004. Microbial nanoparticle production, in Nanobiotechnology, ed. by Niemeyer CM and Mirkin CA. Wiley-VCH, Weinheim.,2004, 126.

16. Shankar SS, Rai A, Ahmad A, Sastry M., 2004. Rapid synthesis of Au, Ag and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J Colloid Interface Sci; 275(2):496-502.

17. Singaravelu, G., Arockiyamari, J., Ganesh Kumar,V. And Govindaraju, K. 2007. A novel extra cellular biosynthesis of monodisperse gold nanoparticles using marine algae, *Sargassum wightii* Greville. Colloids and Surfaces B: Biointerfaces, **57**:97-101